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Research Article

Changes in caddisflies community composition and distribution along 60 years timespan monitoring in Luxembourg

Alain Dohet[1], Sarah Vray[1], Lionel L'Hoste[1]

1 *Environmental Research and Innovation Department, Luxembourg Institute of Science and Technology, Esch-sur-Alzette L-4362 Luxembourg, Luxembourg*

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Corresponding author: Alain Dohet (alain.dohet@list.lu)

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Abstract

In Luxembourg, caddisflies have been systematically collected since the early Sixties. Three periods of exhaustive sampling may be distinguished: the Sixties; 1994 to 2002; and a long period from 2007 to the present time in the frame of the Water Framework Directive. Bearing in mind the uneven sampling procedure across periods, we aim to document changes in community composition and distribution through time including the nature of these changes (e.g. gains and losses of species). We hypothesise different trends of species gains and losses for specialist species in comparison to generalist species. Therefore, we propose a method to identify specialist and generalist species in our dataset.

Historical data (1961 to 1968) lack information on precise locations and abundance of specimen collected. Consequently, cell grids of original distribution maps are used to compare caddisfly community assemblages along the three monitoring periods. We assess the changes that occur on presence/absence data in specific groups of species (i.e. cold-adapted, warm-adapted specialists and generalist species).

Temporal β-diversity results reveal that survey intervals for each monitoring period are dominated by species losses when the comparison is restricted to cold-adapted species. On the other hand, warm-adapted and generalist species are increasing from the Sixties period when compared to the two next periods (1994–2002 and 2007–2020). However, the comparison of the most recent periods reveals species losses even for the warm-adapted and generalist species.

This complex picture of caddisflies species losses and gains in different ways through time, amongst river types and in response to different pressures, is discussed.

Key Words

freshwater ecosystems, long-term time monitoring, macroinvertebrates, specialist and generalist species

Introduction

Scientists all over the world generally agree that we are now entering the sixth mass extinction of species. Amongst ecosystems, freshwaters are one of the most vulnerable to biodiversity loss (Dudgeon et al. 2006; Woodward et al. 2010; Haase et al. 2023). There is some evidence that streams and rivers rank amongst the most threatened freshwater ecosystems due to the combined effects of multiple anthropogenic pressures (Xenopoulos et al. 2005; Ormerod et al. 2010; Domisch et al. 2013).

However, the link between global extinctions and changes in local assemblages remains unclear (Larsen et al. 2018). Although, in recent years, many studies have reported declines in species' richness (e.g. Ormerod et al. (2010); Hallmann et al. (2017, 2020); Powney et al.

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(2019)), some others have documented an increase in freshwater invertebrate abundance (van Klink et al. 2020; Gebert et al. 2022).

It is likely that species with restricted ecological niches (specialists), i.e. species highly specialised to a single habitat or a food source, are more sensitive to large-scale changes than species with broader niches (generalists) (Kotiaho et al. 2005). Long-term persistence of specialist species is adversely affected by past and current global changes (Clavel et al. 2011). However, impacted insect groups not only include specialists, but also common and generalist species (Sánchez-Bayo and Wickhuys 2019).

Amongst freshwater invertebrates, the aquatic insect order Trichoptera (caddisflies) represent a diverse range of biological and ecological traits (Hering et al. 2009).

The first mention of a Trichoptera observation in Luxembourg is dated from the early 20th century (Le Roi 1914). Later, FCJ Ficher (1960–1962) published in his "Trichopterum catalogus" observations of caddisflies collected along a few Luxembourg rivers in 1949. A couple of years later, in the early Sixties, the first nationwide investigation of caddisflies started in the country. During almost 10 years, J. Hoffmann travelled the rivers of Luxembourg looking for caddisflies in every suitable biotope (Hoffmann 1967, 1970a, 1970b). Despite specific limitations (in particular, the absence of precise locations of the sites investigated), this work is of fundamental importance and offers a unique opportunity to study how caddisfly communities have changed over the past six decades in the streams and rivers of the country.

More than 20 years later, in 1994, a first extensive monitoring of freshwater invertebrates was implemented in Luxembourg. For 8 years, from 1994 to 2002, a spatially representative sampling survey, with a particular emphasis on headwater streams, had been set up (Dohet et al. 2002, 2008a, 2008b). Since 2007, benthic invertebrates have been sampled within the scope of the Water Framework Directive (Directive 2000/60/EC) with standardised protocols. Since 2017, the sampling strategy involves a survey of 120 sites comprising a significant proportion of headwater streams.

In this study, we aimed to: (1) compile a list of specialist and generalist caddisfly species, based on their ecological preferences and functional traits and (2) using this list, we want to analyse the long-term temporal changes in their communities.

Following the approach of Hering et al. (2009) and Conti et al. (2014), this paper describes a method to distinguish specialist and generalist species by assigning to each species of our dataset, a selection of various ecological, biological and history traits.

Then we investigate the long-term temporal variation in caddisfly communities by combining this heterogeneous dataset with different sampling methods and spatial scales, into one analysis. To explore this monitoring data along a timespan of more than 60 years, we used the temporal beta□diversity index (TBI) approach developed by Legendre (2019). The objective was to compare observations made during three separate survey periods, involving several sites and to analyse the changes in dissimilarity (beta□diversity) between communities over time. Moreover, we wanted to quantify the extent to which these changes were attributable to community declines ("species losses") or increases ("species gains"). To do so, we grouped taxa according to their ecological preferences and functional traits in specialist species (cold- or warm-adapted species) and generalist species.

We expected to see higher dissimilarities between caddisfly assemblages when comparing historical period (P1) to contemporary ones (P2 and P3) than comparing the two most recent periods.

We anticipated species gains for generalist and warm-adapted species, especially between historical and contemporary periods in response to warming due to climate change. For cold-adapted species, we expected a gradual erosion of their diversity over the 60 years' time span.

Methods

Dataset of Trichoptera monitoring

The dataset of caddisfly monitoring in Luxembourg has been separated in three periods: P1 (1961–1968), P2 (1994–2002) and P3 (2007–2020).

The sampling protocol during P1 was only partially described. Hoffmann (1970a) mentioned that all the biotopes likely to shelter Trichoptera were considered. The inventories were mainly based on the capture and identification of adults, although some larvae and aquatic pupae were captured and raised in the laboratory to be identified at the adult stage. Information on the distribution of caddisflies was defined for each species as "wide" or "restricted", but real abundances values were not available. Sampling places were projected on maps, using a 10-km UTM grid according to the work of Stroot (1984).

During the second period (i.e. P2), a total of 239 sites, distributed all over the country, were sampled twice a year (spring and summer-autumn seasons), in the different microhabitats (riffles, depositional zones, different types of vegetation). Inventories were mainly based on Trichoptera larvae, but were completed by light trap sampling of adults during one season in each site. Caddisflies were identified at a species level and abundances recorded in individuals per m^2. The sampling protocol was described in Dohet et al. (2002).

During the most recent period (i.e. P3), caddisflies were collected within the scope of the Water Framework Directive (Directive 2000/60/EC). During a first phase (2007–2017), 20 sites were monitored each autumn according to a standardised multi-habitat sampling protocol for benthic invertebrates (AFNOR 2010, 2015, 2016). From 2017, the survey was extended to 120 sites, using a stratified random sampling over different stream types, ecoregions and Natura 2000 delineations (Directive 92/43/EC).

A subset of spring and mainly autumn samples were monitored each year following the same protocol.

A total of 200 caddisflies species have been observed in Luxembourg since the first inventories in the Sixties. After removing species living exclusively in lentic waters (e.g. reservoirs, ponds, wetlands) and species recorded outside the defined periods, a total of 124 caddisfly species was considered in our dataset for subsequent analysis. This represents 12,045 occurrences of caddisflies: 912 in P1, 5056 in P2 and 6077 in P3. Since abundance data and precise locations of the sampling sites were not available for the first period (P1), we used presence/absence data of caddisflies collected along the three periods and the 10-km UTM grid cells as geographic units for analyses.

Identification of specialist and generalist species

To classify caddisflies as specialist or generalist species, we selected a wide range of various ecological, biological and life history traits. These ecological preferences and biological characteristics were extracted from the open-access database www.freshwaterecology.info (Graf et al. 2008, 2019; Schmidt-Kloiber and Hering 2015). In this database, the occurrence of different trait modalities was coded as presence/absence, single category assignment or by fuzzy coding using a 10-point assignment system (see www.freshwaterecology.info for more information on the coding system applied). The general hypothesis governing the selection of traits was that an extensive functional space covering a wide range of ecological and biological characteristics will provide a more precise estimation of the specific requirements of the species and will enable the appropriate classification of specialist and generalist species in our dataset. For several ecological parameters, limited information is available. In order to maximise the number of species and the trait coverage across species, we only selected parameters coded for at least 50% of the species of our dataset. Finally, a total of nine parameters including 51 trait modalities was considered: stream zonation preference; hydrologic preference; microhabitat/substrate preference; feeding type; habitat specialist; temperature range preference; climate change vulnerability score; and duration of emergence period/reproductive life cycles per year. Detailed information on the assignment system and the method used to calculate single scores for each ecological/biological parameter are given in Table 1.

To address the potential impact of changing climate, several parameters enabling us to detect and evaluate temperature increases as induced by climate change, were selected. The parameter "temperature range preference" allows us to separate, amongst specialist taxa, the cold-adapted caddisfly species (i.e. cold-stenotherms) from one hand and the warm-adapted species (i.e. warm-stenotherms) from the other hand. In our proposed method, a score of "10" is given to species coded as cold-stenotherm or warm-stenotherm in the www.freshwaterecology.info database. Moreover, the parameter "climate change vulnerability score", calculated from six autecological traits that are known to be associated with vulnerability to climate change (i.e. endemism, micro-endemism, temperature preference, altitudinal preference, stream zonation preference and life history (Hershkovitz et al. 2015), is added to calculate the score for each caddisfly species (Table 1).

Table 1. Ecological parameters selected from www.freschwaterecology.info, assignment system used (10p: ten points assignment system, s.c: single assignment system), percentage number of classified Trichoptera from our dataset, and calculation method of a single score for each parameter (HV - nC : higher value in a category minus number of categories with values ≥ 1, 0/5 and 0/10: 5 or 10 points if the taxa is coded as "1" in the single category assignment system, 0 if not, 0–10: 0–6: 0 low vulnerability to 6 high vulnerability to climate change, re-scaled to 1–10 value).

Parameter	Assignment system	Percentage of classified taxa	Calculation
Stream zonation preference	10p	99	HV - nC
Hydrologic preference	10p	75	HV - nC
Microhabitat/substrate preference	10p	88	HV - nC
Habitat specialist	s.c	62	0 / 5
Feeding type	10p	90	HV - nC
Temperature range preference	s.c	62	0 / 10
Climate change vulnerability score	metric value (0–6)	58	0 - 10
Duration of emergence	s.c		0 / 5
period / Reproductive life cycles per year	s.c	75	0 / 5

Finally, a mean score is calculated for each species from the nine parameters selected (Tables 2, 3 and 4). The distribution of the mean-scores' values are calculated separately for cold-adapted and warm-adapted species. Only the species showing a score higher than the 75 percentile of each distribution values are selected as specialist species, either cold-adapted or warm-adapted. The distribution of the mean-scores' values calculated for all species of our dataset enables to select generalist species having mean scores lower than the 25 percentile of the "all species" distribution values.

Temporal beta-diversity index

Changes in the composition of caddisfly communities amongst the three periods (P1, P2 and P3) were assessed using the temporal beta□diversity index (TBI) approach developed by Legendre (2019). The TBI is a dissimilarity index measuring the change in community composition amongst several geographic units within the same region, i.e. measuring the change in beta-diversity, between two separate surveys over time. We compared the

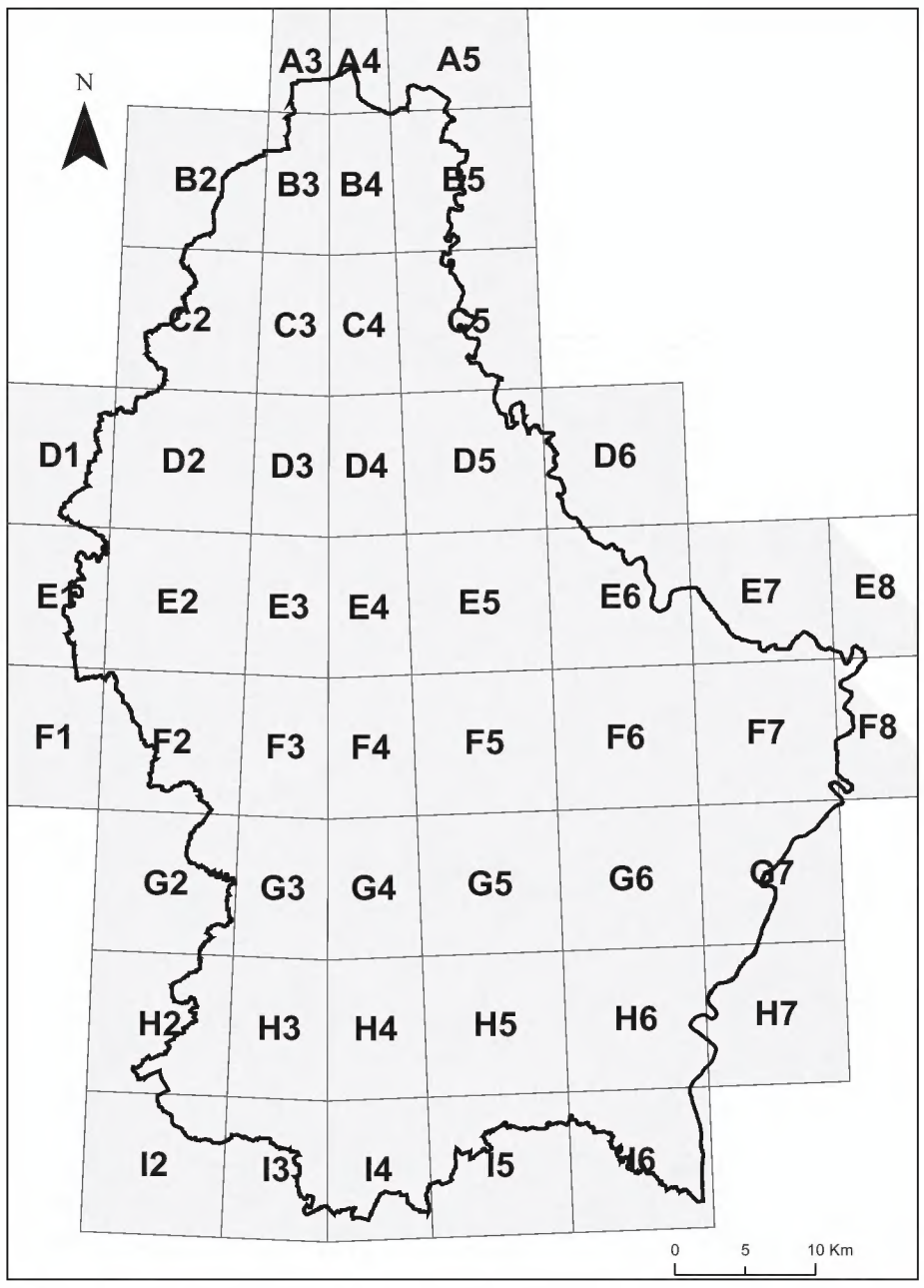

Figure 1. Map of the 10-km UTM grid of Luxembourg.

three periods two by two, resulting in three analyses. As data from P1 were at the resolution of the 10-km UTM grid and consisted of presence-absence only, we used the UTM grid cells as geographic units (Fig. 1) and the Jaccard index to compute the TBI. The Jaccard index is an occurrence-based (i.e. binary) coefficient using only the double-presences in the calculation of dissimilarity, i.e. its value does not change when double-zeroes are added to the data (Legendre and De Cáceres 2013). This index, therefore, considers only the species present at least in one of the two periods.

We measured the dissimilarity in species composition using the TBI, based on the Jaccard index in each grid cell, between the first (i.e. P1 or P2) and the second (i.e. P2 or P3) periods. We then decomposed each TBI index into two finer indices: one for species losses (B) and one for species gains (C). B > C or B < C means that the grid cell has lost or gained species between the two periods, respectively. Grid cells with equal amounts of losses and gains would have B equal to C. We used the B and C indices to produce B-C plots, with B (losses per grid cell) in the abscissa and C (gains per grid cell) in the ordinate, as described in Legendre (2019). These plots display the relative importance of species losses and gains across the grid cells, where the green line indicates the theoretical positions of grid cells with equal amounts of losses and gains and the parallel red line indicates the centroid of all grid cells. Therefore, a red line below the green line indicates a dominance of species losses across the grid cells, while a red line above the green line indicates a dominance of species gains. Distinctive symbols are used for the grid cells dominated by gains (squares) and by losses (circles) and the symbol diameter represents the TBI value. To have an overall indication of the direction of change over all grid cells, we tested the difference between species gains and losses using a permutation paired t-test with 9999 random permutations. All calculations and plots were made using the "TBI" and "plot.TBI" functions available in the R package adespatial (Dray et al. 2019).

Table 2. Selected cold-adapted species, trait modalities of species with mean scores "TM_Mean" higher than the 75 percentile of the values' distributions for all "cold-adapted" classified species ("Therm –" = 10). "Zon": Zonation preference; "Hydr": Hydrological preference; "Habit": Habitat preference; "Habit S": Habitat specialist; "Feed": Feeding preferences; "CCV": Climate Change Indicator; "SEP": Short Emergence Period; "UNV": Univoltine. * Species only present during the historical period (P1).

	Zon	Hydr	Habit	Habit S	Therm -	Therm +	Feed	CCV	SEP	UNV	TM_Mean
Apatania fimbriata	4	0	3	5	10	0	6	9	0	5	**6.00**
Beraea maurus	7	9	9	5	10	0	5	9	0	0	**7.71**
Ernodes articularis *	7	9	9	5	10	0	3	9	5	0	**7.13**
Agapetus fuscipes	7	9	3	5	10	0	6	6	0	0	**6.57**
Agapetus ochripes	4	9	3	5	10	0	6	0	5	0	**6.00**
Glossosoma boltoni	2	9	3	5	10	0	6	0	0	0	**5.83**
Synagapetus dubitans	7	0	3	5	10	0	6	6	0	0	**6.17**
Synagapetus iridipennis	7	0	3	5	10	0	6	6	0	0	**6.17**
Lithax niger	5	0	3	5	10	0	7	6	5	0	**5.86**
Diplectrona felix	5	9	-1	0	10	0	0	9	0	5	**6.17**
Adicella filicornis *	3	9	6	5	10	0	6	9	5	5	**6.44**
Wormaldia occipitalis occipitalis	3	9	3	5	10	0	9	9	0	0	**6.86**
Wormaldia occipitalis subterranea	0	0	3	5	10	0	0	6	0	0	**6.00**
Plectrocnemia brevis	3	0	-3	0	10	0	7	6	0	0	**7.00**
Tinodes unicolor	6	0	3	5	10	0	5	6	0	0	**5.83**
Ptilocolepus granulatus	9	9	9	5	10	0	3	9	0	5	**7.38**
Rhyacophila laevis	9	0	3	5	10	0	9	6	5	0	**6.71**
Rhyacophila philopotamoides	3	0	3	5	10	0	9	6	5	0	**5.86**
Rhyacophila pubescens	6	0	3	5	10	0	9	6	5	0	**6.29**

Table 3. Selected warm-adapted species, trait modalities of species with mean scores "TM_Mean" higher than the 75 percentile of the values' distributions for all "warm-adapted" classified species ("Therm +" = 10). "Zon": Zonation preference; "Hydr": Hydrological preference; "Habit": Habitat preference; "Habit S": Habitat specialist; "Feed": Feeding preferences; "CCV": Climate Change Indicator; "SEP": Short Emergence Period; "UNV": Univoltine. * Species only present during the historical period (P1).

Species	Zon	Hydr	Habit	Habit S	Therm -	Therm +	Feed	CCV	SEP	UNV	TM_Mean
Agapetus laniger *	3	9	3	5	0	10	6	0	0	0	**6.00**
Goera pilosa	2	7	3	5	0	10	7	0	5	0	**5.57**
Silo nigricornis	-1	9	3	5	0	10	7	0	0	0	**5.50**
Cheumatopsyche lepida	3	9	0	0	0	10	2	3	0	5	**5.33**
Hydroptila angulata	0	6	9	5	0	10	0	0	0	0	**6.00**
Hydroptila lotensis	1	4	9	5	0	10	0	0	0	0	**5.80**
Hydroptila simulans	0	3	9	5	0	10	0	0	5	0	**5.33**
Orthotrichia costalis	9	4	0	0	0	10	9	0	0	0	**8.00**
Oxyethira flavicornis	1	0	9	0	0	10	9	0	0	0	**5.80**
Ceraclea fulva	-1	9	3	5	0	10	9	3	0	5	**5.38**
Leptocerus interruptus	1	9	9	5	0	10	3	3	5	5	**5.56**
Cyrnus flavidus	1	6	7	5	0	10	7	0	5	0	**5.86**
Tinodes pallidulus	4	9	0	5	0	10	5	0	0	0	**5.50**
Rhyacophila dorsalis dorsalis	3	9	3	5	0	10	9	3	0	5	**5.88**
Rhyacophila fasciata fasciata	4	9	3	5	0	10	9	0	0	0	**6.67**

These analyses were performed for the three caddisfly ecological groups (i.e. the cold-adapted species, the warm-adapted species and the generalist species).

Results

Functional traits modalities and ecological preferences

The proposed method to identify specialist and generalist species and to distinguish, amongst specialists, the species which are cold-adapted from those that are warm-adapted, resulted in a classification of 32 species (36% of the species in this dataset). Split up in distinct categories, 9.5% of species were classified as cold-adapted species, 7.5% as warm-adapted and finally 19% as generalist species (Tables 2, 3 and 4).

Values assigned to the nine selected parameters are summarised in Table 1 for cold-adapted species, in Table 2 for warm-adapted species and in Table 3 for generalist species. For cold (Table 2) and warm-adapted species (Table 3), only species with respectively "Therm – = 10" and "Therm + = 10" are classified. Only those with "TM-Mean" higher than the 75th percentile of their value's distributions are indicated (5.71 for cold-adapted species and 5.29 for warm-adapted species, respectively). For generalist species (Table 4), all species having a "TM-Mean" value lower than 3.50 of the all-species value's distribution (25th percentile) are indicated.

Changes in Trichoptera community composition through time

The results from the TBI analyses comparing the three periods two by two are summarised in Table 5 and are illustrated in the B-C plots in Figs 2, 3 and 4 for cold-adapted species, warm-adapted species and generalist species, respectively.

For each period comparison, changes in communities of cold-adapted species tend to be dominated by species losses, although none of the paired t-tests shows a significant change ($p > 0.05$; Table 5). Nevertheless, this tendency to species loss is higher when comparing P1 with P2 (Fig. 2a) and P1 with P3 (Fig. 2b) than when comparing the two last periods (Fig. 2c), as indicated by the red line being further below the green line on the B-C plots.

Concerning the changes in communities of warm-adapted species (Fig. 3), although not significant ($p > 0.05$), the tendency is opposite when comparing the historical period (P1) to the recent periods P2 (Fig. 3a) and P3 (Fig. 3b), with grid cells generally dominated by species gains. On the contrary, the most recent changes (i.e. between P2 and P3) were more dominated by species losses as for cold-adapted communities (Fig. 3c)), although this change is not significant ($p > 0.05$).

Changes in the community composition of generalist species follow the same tendencies as changes in warm-adapted communities, but with significant trends (Table 5). Between P1 and P2, changes are significantly dominated by species gains (Fig. 4a), whereas they are significantly dominated by species losses between P2 and P3 (Fig. 4c).

The maps of the Fig. 5 illustrate the geographical distribution over the three periods of an example of a cold-adapted caddisfly species, *Lithax niger* (Fig. 5a), a warm-adapted species, *Leptocerus interruptus* (Fig. 5b) and a generalist species, *Allogamus auricollis* (Fig. 5c).

Discussion

List of specialist (cold and warm-adapted) and generalist species

The relatively large number of species listed as specialists (17%, summing cold and warm-adapted species) confirms the high potential of caddisflies as a species-rich and ecologically diverse insect order to reflect the intensity of

Table 4. Selected generalist species, trait modalities of species with mean scores "TM_Mean" lower than the 25 percentile of the values' distributions for all species. "Zon": Zonation preference; "Hydr": Hydrological preference; "Habit": Habitat preference; "Habit S": Habitat specialist; "Feed": Feeding preferences; "CCV": Climate Change Indicator; "SEP": Short Emergence Period; "UNV": Univoltine. * Species only present during the historical period (P1).

Species	Zon	Hydr	Habit	Habit S	Therm -	Therm +	Feed	CCV	SEP	UNV	TM_Mean
Beraeodes minutus	-1	-1	1	0	0	0	9	0	5	0	**2.60**
Brachycentrus maculatus	4	0	1	5	0	0	0	0	5	0	**3.00**
Brachycentrus montanus	2	0	1	5	0	0	2	3	5	0	**3.00**
Hydropsyche angustipennis	-1	6	-1	0	0	10	2	3	0	5	**3.43**
Hydropsyche botosaneanui	0	0	-1	0	0	0	2	0	5	0	**1.50**
Hydropsyche bulgaromanorum	-1	4	-1	0	0	0	2	0	0	0	**1.00**
Hydropsyche dinarica	2	0	-1	0	0	0	2	0	0	0	**1.00**
Hydropsyche exocellata	1	9	-1	0	0	0	2	0	0	0	**2.75**
Hydropsyche incognita	2	9	-1	0	0	0	2	0	0	0	**3.00**
Orthotrichia angustella	-4	6	0	0	0	0	0	0	5	0	**2.33**
Mystacides azurea	-1	1	0	0	0	10	1	3	0	5	**2.71**
Mystacides longicornis	3	-1	0	0	0	10	1	3	0	5	**3.00**
Mystacides nigra	-1	1	0	0	0	10	1	3	0	5	**2.71**
Annitella obscurata	4	0	-3	0	0	0	3	0	5	0	**2.25**
Chaetopteryx villosa	-4	5	0	0	0	10	0	3	0	5	**2.71**
Limnephilus affinis	1	-1	0	0	0	10	2	0	0	0	**3.00**
Limnephilus centralis	1	3	0	0	0	0	2	3	0	0	**2.25**
Limnephilus ignavus	0	-1	0	5	10	0	2	3	0	0	**3.17**
Limnephilus sparsus	-3	1	2	5	0	10	2	0	0	0	**2.83**
Allogamus auricollis	-1	6	-1	0	0	10	-1	3	5	0	**3.00**
Hydatophylax infumatus	1	0	3	5	0	0	4	0	0	0	**3.25**
Melampophylax mucoreus	1	0	-1	0	0	0	5	0	5	0	**2.50**
Micropterna lateralis	1	0	0	0	0	0	3	3	0	0	**1.75**
Micropterna sequax	1	0	3	0	0	0	3	3	0	5	**3.00**
Micropterna testacea	1	0	0	0	0	0	3	0	0	0	**2.00**
Parachiona picicornis *	4	0	-2	5	0	0	1	3	5	0	**2.67**
Potamophylax latipennis latipennis	-4	6	1	0	0	0	3	3	0	0	**1.80**
Potamophylax luctuosus luctuosus	1	6	1	0	0	0	3	0	5	0	**3.20**
Stenophylax mitis	1	0	0	0	0	0	0	0	0	0	**1.00**
Stenophylax mucronatus	1	0	0	0	0	0	0	0	0	0	**1.00**
Stenophylax vibex	1	3	0	0	0	0	0	0	0	0	**2.00**
Plectrocnemia conspersa	0	4	-3	0	0	0	7	3	0	5	**2.67**
Polycentropus flavomaculatus	-3	3	-3	0	0	10	7	3	0	5	**3.14**
Polycentropus irroratus	3	3	-3	0	0	0	7	0	0	0	**2.50**
Tinodes assimilis	-3	0	3	5	0	0	5	0	0	0	**2.50**
Tinodes maculicornis	-5	6	3	5	0	0	5	0	0	0	**2.80**
Sericostoma personatum	2	9	-4	0	0	0	7	3	0	0	**3.40**
Sericostoma flavicorne	-1	0	-4	0	0	0	7	3	0	0	**1.25**

different stressors on aquatic ecosystems (Hering et al. 2009; Conti et al. 2014).

In each category, several species are nowadays considered as very or extremely rare. We can particularly mention *Ernodes articularis* and *Adicella filicornis* (only observed during the Sixties and now probably extinct in the country), *Lithax niger*, *Plectrocnemia brevis* and *Rhyacophila philopotamoides* amongst the cold-adapted species; *Agapetus laniger* (only observed during the Sixties and probably now extinct in the country), *Hydroptila angulata*, *Hydroptila lotensis*, *Orthotrichia costalis*, *Oxyethira flavicornis*, *Ceraclea fulva* and *Leptocerus interruptus* amongst the warm-adapted species. Even amongst generalists, numerous species show moderate to strong decrease during the recent years, like *Brachycentrus montanus*, *Hydropsyche botosaneanui*, *Hydropsyche dinarica*, *Annitella obscurata* or *Tinodes assimilis*. Some other generalist taxa have always been very rare in the area, for example, *Hydropsyche bulgaromanorum*, *Hydropsyche exocellata*, *Orthotrichia angustella*, *Limnephilus ignavus*, *Limnephilus sparsus*, *Stenophylax mitis* or *Stenophylax mucronatus*. *Parachiona picicornis* is probably extinct. We should, however, mention that several species enumerated here as extremely rare or extinct species nowadays are also characteristic of very specific habitats (e.g. springs, swampy springs) or, can hardly be identified at larval stages (e.g. Hydroptilidae). Therefore, they are likely to be absent from usual monitoring lists if these specific habitats are not sufficiently monitored or if adults or aquatic pupae are not found in benthic samples.

Temporal trend of changes within caddisfly assemblages over 60 years

In this study, we analysed the long-term temporal changes of caddisfly communities in Luxembourg. We compared the changes that occurred in specific groups of species

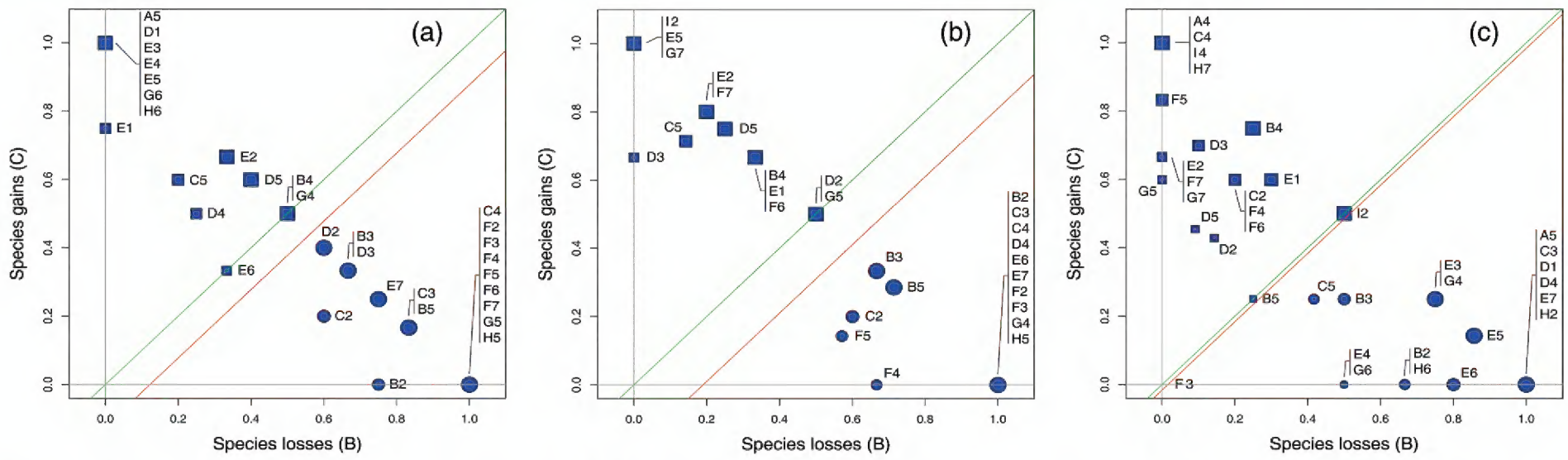

Figure 2. B-C plots for species presence/absence data of cold-adapted species, **a.** Comparison of Period 1 and Period 2; **b.** Comparison of Period 1 and Period 3; **c.** Comparison of Period 2 and Period 3. Sites (cells grids of the UTM projected distribution map of Luxembourg) are identified by squares when the sites are dominated by species gains and by circles when they are dominated by losses. The symbols are drawn to sizes representing the values statistics. The diagonal green line, with a slope of 1, drawn through the origin, represents the theoretical positions of sites where the gains equal to the losses. The red line drawn parallel to the green line and passing through the centroid of the points. Its position below the green line indicates that, on average, species losses dominated gains between Period 1 and Period 2 (**a**), between Period 1 and 3 (**b**) and between Period 2 and 3 (**c**).

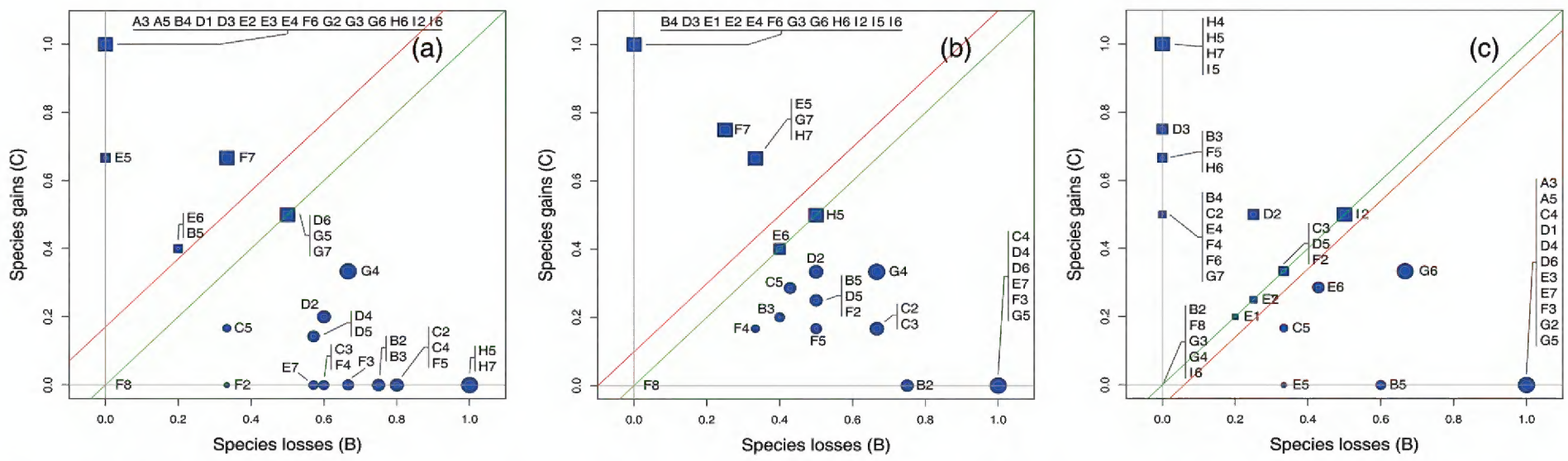

Figure 3. B-C plots for species presence/absence data of warm-adapted species, **a.** Comparison of Period 1 and Period 2; **b.** Comparison of Period 1 and Period 3; **c.** Comparison of Period 2 and Period 3. For green and red line details, see legend of Fig. 2.

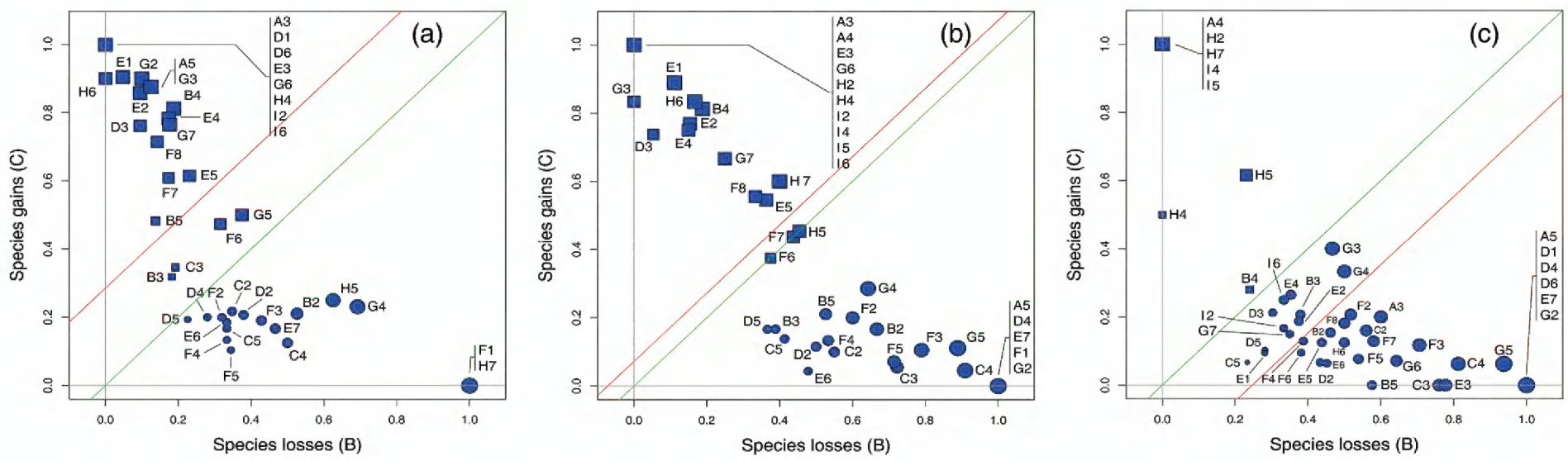

Figure 4. B-C plots for species presence/absence data of generalist species, **a.** Comparison of Period 1 and Period 2; **b.** Comparison of Period 1 and Period 3; **c.** Comparison of Period 2 and Period 3. For green and red lines details, see legend of Fig. 2.

(i.e. specialist and generalist species) that are known to react differently to environmental stressors (Hering et al. 2009; Conti et al. 2014).

As expected, the overall change in species composition between periods for cold-adapted species was dominated by a loss of species (Table 5, Fig. 2). This general trend was more preeminent when comparing the historical and contemporary data than when comparing the two most recent periods. Numerous studies reported clear benthic invertebrate's declines, especially when specialist or cold-adapted species were considered (Durance and Ormerod 2007; Domisch et al. 2011, 2013; Conti et al. 2014; Dohet et al. 2015; Haase et al. 2019). A large-scale loss of the potential distribution ranges of the entire cold-stenothermic aquatic insect assemblages has been predicted by the end of this century (Sauer et al. 2011).

Even though, long-term datasets covering more recent periods have also related stabilisation of cold-adapted species richness in montane and subalpine/alpine streams (Gebert et al. 2022). These results align with those

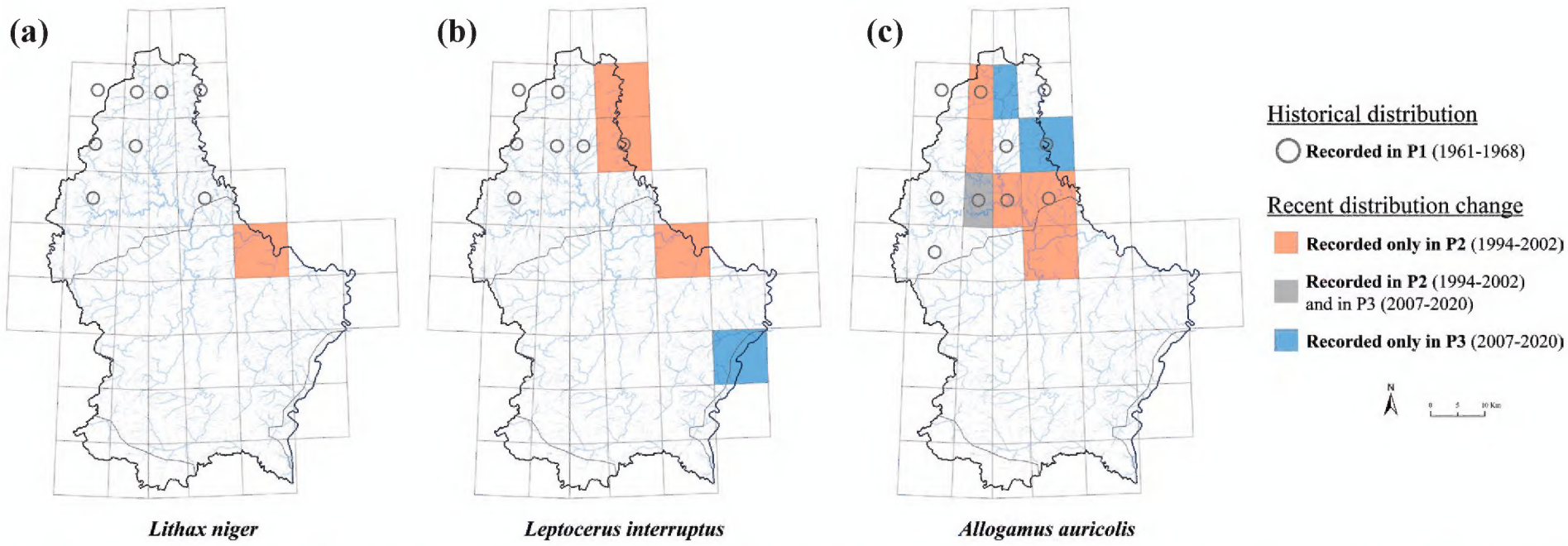

Figure 5. Distribution maps of a cold-adapted caddisfly species (*Lithax niger*), a warm-adapted species (*Leptocerus interruptus*) and a generalist species (*Allogamus auricollis*).

Table 5. Summary of the TBI analysis results. The mean dissimilarity index is the mean of the Jaccard indices over all grid cells, which gives an estimate of the overall change in species composition between the two periods. "+" and "–" indicate which of the processes dominated the change, i.e. gain or loss of species, respectively. The difference between species gains and species losses is tested in a permutation paired t-test. "*" indicates a significant change (p-value ≤ 0.05).

TBI analysis results	P1 – P2	P1 – P3	P2 – P3
Cold-adapted species			
Mean dissimilarity index	0.954	0.954	0.797
Change	–	–	–
p-value from the paired t-test	0.375	0.190	0.889
Warm-adapted species			
Mean dissimilarity index	0.836	0.886	0.676
Change	+	+	–
p-value from the paired t-test	0.151	0.423	0.562
Generalist species			
Mean dissimilarity index	0.797	0.878	0.719
Change	+	+	–
p-value from the paired t-test	0.002*	0.491	0.005*

indicating that freshwater insects, contrary to terrestrial insects, have frequently been reported increasing in abundance within these later years (van Klink et al. 2020). This current state of increasing taxonomic richness has been interpreted as a transient condition that could eventually result in the local extinction of cold-adapted species and the prevalence of more generalist warm-adapted and eurytherm species, which are better adapted to the new climatic conditions (Steinbauer et al. 2018; Haase et al. 2019; Gebert et al. 2022).

In the present dataset, the temporal trends of changes within warm-adapted species mirrored those of the generalist species (Table 5, Figs 3 and 4). Between historic period and contemporary periods, changes in warm-adapted and generalist communities were dominated by species gains, whereas they were dominated by species losses between the two contemporary periods. The concomitant decline of cold-adapted species and increase in species adapted to higher temperatures and generalist species suggests the replacement of local species by other, more widespread species (Clavel et al. 2011; Haase et al. 2019). This process can potentially lead to novel assemblages which could affect community dynamics, through, for example, biotic and functional homogenisation of communities and ultimately biodiversity maintenance and ecosystem functioning (Clavel et al. 2011; Gebert et al. 2022). However, during the last decades (comparison of P2 and P3: Figs 3c and 4c), changes in the community composition of generalist species and warm-adapted species were dominated by species losses. This result suggests that affected insect groups not only include specialists that occupy particular ecological niches, but also common and generalist species (Sánchez-Bayo and Wickhuys 2019). In contrast, on a comparable timespan and in the same region (central Europe), Haase et al. (2019) reported profound changes in stream invertebrate communities. A warming of 0.5 °C over the past 25 years was associated with significant increases in total abundance and richness of warm-adapted and eurytherm species (Haase et al. 2019). This pattern was interpreted as an increase in primary productivity induced by slight increases in temperature in these streams, which provides additional food resources and niche space for invaders, enabling a higher abundance and richness of consumer species (Haase et al. 2019). The decline of cold-adapted species and increase in species preferring higher temperatures suggests a similar underlying process as the one found by Steinbauer et al. (2018) with repeated plant surveys from mountain summits across Europe. Shifts in relative abundance were considered as early warning signals of upcoming local extinctions because competitive replacement of resident species required that colonisers build up sufficiently large populations. Local extinctions should, hence, follow colonisation with a time-lag (Steinbauer et al. 2018).

Long time-series datasets, like the one available in Luxembourg for caddisflies, are paramount to predict future trends and to better understand trajectories and the degree of change of biodiversity (Larsen et al. 2018). Though, our dataset was subject to several constraints. In particular, the lack of abundance data for the historical period limits our interpretation of the comparison of species losses and gains within the three periods analysed in this study. Species' abundance is potentially more sensitive to global change than a binary presence/

absence change and understanding the causes of changes in species' abundances is crucial to assess consequences for ecosystem functioning and extinction risk (Bowler et al. 2017). Furthermore, the sampling methods and study designs changed between the three study periods, which could have influenced our results. Particularly, the lack of homogeneity in sampling seasonality and spatial coverage between periods does not allow us to draw definitive conclusions on the reliable absence of either species in each grid cell and period. Indeed, the absence of a species could result from an insufficient sampling effort in a particular region or stream type and/or because the survey effort was restricted to a particular season which did not enable the specific identification of the larval stages of this species. Finally, if specialist and generalist benthic invertebrates can be regarded as reliable integrative indicators of water temperature fluctuations, overriding effects of different other stressors (e.g. land use, eutrophication, siltation, toxic substances, invasive species) should not be neglected when interpreting long-time series dataset.

To remediate to these weaknesses, our caddisfly dataset will be completed with a new three-year sampling cycle (2021–2023) in the near future. These complementary data will partly solve the discrepancies in sampling coverage and seasons between the two more recent study periods. When accounted for the differences in sampling procedures and design, the abundance of caddisflies will be used for a better understanding of the temporal trends of changes within communities between the more contemporary periods. Finally, temporal beta-diversity analysis will be tested to the subgroup of sites corresponding to the different stream types present in Luxembourg (Ferréol et al. 2005). We postulate that the evaluation of species losses and gains amongst stream types along a sixty years' timespan will enable us to investigate the influence of the upstream–downstream gradient in structuring communities. Elevational range shifts may be especially important for stream invertebrates because streams and their resident communities follow a natural elevation gradient from source to mouth and stream organisms like caddisflies, have higher dispersal capacities within compared to between river networks (Haase et al. 2019).

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